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Molecular Cloning of *Actinomyces* Bacteriophage DNA in *E. coli*

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ABSTRACT

Actinomyces are gram-positive filamentous bacteria that colonize tooth and mucosal surfaces and coaggregate with other bacteria to initiate plaque formation in the oral cavity. Bacteriophages that cause cell lysis in strains of *Actinomyces* have been isolated from dental plaque samples. The identification of a phage gene encoding host cell lysis activity may provide a means for the design of strategies that inhibit plaque formation. The purpose of this study was to initiate molecular cloning of DNA fragments from an *Actinomyces* lytic phage, designated ϕ63, that contained a putative lysin gene. Purified ϕ63 genomic DNA was digested with *Eco*RI, and DNA fragments of 10-, 2- and 1.8- kilobasepairs (kb) were subcloned onto the expression vector, λgt11. This vector allows color selection of recombinant clones, which appear as colorless plaques on a medium containing the chromagen, X-gal, versus non-recombinant clones which appear blue. Three libraries consisting of the ϕ63 DNA fragments in λgt11 were obtained and the cloning efficiencies ranged between 10^3 and 10^6 plaque forming units per milliliter of recombinant phages. Analysis of randomly selected recombinant clones revealed the presence of the expected ϕ63 DNA fragments that were used in the subcloning and they were stably maintained in *E. coli*. Further analysis of the inserted DNA fragments will indicate whether they contain a putative lysin gene. Importantly, the results of this study indicate the feasibility of cloning of *Actinomyces* phage DNA fragments onto an *E. coli* expression vector.

INTRODUCTION

Actinomyces are gram-positive filamentous bacteria that colonize the human oral cavity, attaching to the mucosal and tooth surfaces and cooaggregating with other plaque bacteria via cell surface fimbriae.[1-4] A genetic analysis of the cell surface fimbriae will provide insight into the molecular mechanism of bacterial adherence. Results of these studies may allow the design of appropriate and effective means to modulate plaque formation. The availability of suitable genetic tools, including cloning vectors for *Actinomyces* spp., are crucial to manipulations of genes from these organisms. In this regard, results of a recent study have identified a plasmid that replicates in strains of *Actinomyces* spp.[5] To facilitate further study of *Actinomyces* genes, additional cloning vectors are not only desirable, but necessary.

Results from a previous study indicated that lytic bacteriophages were present in dental plaque samples.[6] Data from a recent study demonstrated that both temperate and lytic bacteriophages that infected *Actinomyces* spp. were isolated from fresh plaque samples.[7] Bacteriophage DNA has been used to develop cloning vectors in several bacterial genera.[8-12] Thus, investigation of Ac*tinomyces* bacteriophages will provide the basis for: 1) the engineering of a bacteriophage-based cloning system for analysis of *Actinomyces* genes; and 2) the isolation and characterization of certain genes, including the lysin gene, that may be useful in developing methods that interfere with bacterial colonization, thereby inhibiting plaque formation in the oral cavity.

This study described further characterization of a lytic *Actinomyces* bacteriophage, designated ϕ63 (Figure 1). Data from a previous study indicated that this bacteriophage is a double-stranded circular molecule of approximately 18 kilobasepairs (kb).[7] A partial restriction endonuclease map for the genome

of this phage was determined and molecular cloning of its DNA fragments into an *E. coli* expression vector was initiated. A preliminary study was conducted to identify a putative lysin gene in the recombinant clones. In addition, a pilot attempt was initiated to detect a lysin gene in the ϕ63 genome by Southern blot hybridization using the *Lactobacillus gasseri* ϕadh lysin gene[13] as a DNA probe.

MATERIALS AND METHODS

Bacteria, plasmids and bacteriophages. The bacterial strains, plasmids and bacteriophages used in this study are summarized in Table 1.

Media and growth conditions. *A. viscosus* strain MG-1 was grown in a complex medium containing trypticase yeast neopeptone and phosphate [TYNP, per liter contained 30 g trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD), 5 g neopeptone (Difco Laboratories, Detroit, MI), and 5 g K_2HPO_4].[6] *E. coli* strains MC 4100 and Y1088 were grown in Luria-Bertani (LB) broth containing 0.2% maltose and 10 mM $MgSO_4$. Streptomycin sulfate or ampicillin at a final concentration of 50 μg/ml was added to the medium for these strains, respectively. *E. coli* DH5α carrying the plasmid pUC19*holys*[13] was grown in LB containing ampicillin at a concentration of 100 μg/ml.

Preparation of bacteriophage lysates. To prepare phage lysates from infected bacteria on an agar plate, an aliquot (100 μl) of a stationary phase culture (optical density at 660 nm ≅ 0.7) of *A. viscosus* MG-1 was mixed with 3 ml of 0.6% Bactoagar in TYNP broth. Bacteriophage particles (10^2 plaque forming units) of ϕ63 were added and the mixture poured over a brain heart infusion agar (BHIA) plate. After overnight incubation at 37°C, phage particles from the agar surface were eluted with TYNP containing 0.1% Tween 20 (Sigma Chemical Co., St. Louis, MO), as described previously, [7] and chloroform was added to stabilize the phage particles. To determine the titer of each plate amplified lysate, dilutions of phage lysates were mixed with *A. viscosus* MG-1 and 0.6% Bactoagar in TYNP as described above, and the number of plaques enumerated. To prepare phage lysates from infected bacteria in a broth culture, various amounts of phage particles were used to infect 100 ml of *A. viscosus* MG-1 in TYNP at the mid-exponential phase of growth (optical density at 660 nm ≅ 0.2). The infected cultures were incubated at 37°C overnight. The supernatant fluid of each culture were collected by centrifugation and the titers determined as described previously.

Isolation of bacteriophage DNA. A mid-exponential phase culture (optical density at 660 nm ≅ 0.2) of *A. viscosus* MG-1 in TYNP was infected with phage particles (approximately 10^9 pfu/ml). After overnight incubation, the culture supernatant fluid was brought to 50% saturation with solid ammonium sulfate, as described previously.[14] Phage particles in the precipitates were suspended in a solution consisting of 3 M guanidine isothiocyanate, 2.5% sarkosyl and 10 mM EDTA, pH 7.5, and digested with proteinase K (200 μg/ml) at 52°C for 30 minutes. The released nucleic acid was digested with RNAse AI (50 μg/ml) and purified by extraction with organic solvents. The isolated phage DNA was suitable for restriction endonuclease analysis.

Restriction endonuclease digestion of bacteriophage DNA. Phage DNA was digested with various restriction endonucleases (GIBCO/BRL Life Technologies Inc., Gaithersburg, MD) in various combinations and the digested DNA fragments were separated by agarose gel electrophoresis in Tris-borate-EDTA buffer .[15] To obtain isolated DNA fragments, 10-20 μg of purified phage DNA was digested with the appropriate restriction endonucleases, and the digested DNA fragments were separated on a 0.6% agarose gel. Selected DNA fragments were extracted from agarose with reagents from the Elu-Quik DNA Purification Kit (Schleicher & Schuell, Keene, NH).

Cloning of bacteriophage DNA. Aliquots of *Eco*RI digested ϕ63 DNA fragments were mixed with λgt11[16], digested with *Eco*RI and dephosphorylated, in the presence of T4 DNA ligase and the mixtures incubated at 14°C. Aliquots of the ligation mixtures were added to *E. coli* cell extracts (Stratagene Cloning Systems, La Jolla, CA), and the procedure of in-vitro recombinant lambda phage packaging was as recommended by the manufacturer. Dilutions of the packaged mixtures were used to transfect *E.* coli Y1088 or MC4100 (Table 1). To transfect strain Y1088, bacteria and packaged phages were incubated at 37°C for 15 minutes and then mixed with LB containing 0.6% Bactoagar, 5-bromo-4-

chloro-3-indoyl-β-D-galactopyranoside (X-gal) and isopropyl-β-D-thiogalactopyranoside (IPTG). The entire content was poured over LB agar plates and incubated at 37°C overnight. A similar procedure was used for transfection of *E. coli* MC4100, but no X-gal nor IPTG was added to the medium.

Analysis of *E. coli* recombinant plaques. Phage particles in agar plugs were eluted in 500 µl of SM buffer (0.1 M NaCl, 50 mM Tris, pH 7.5, 10 nM $MgSO_4$, 0.1% gelatin) overnight at 4°C. Thirty microliters of the eluted phage lysate were mixed with *E. coli* Y1088 (100 µl of an overnight culture in LB), incubated at 37°C for 20 minutes and then diluted with 10 ml of LB supplemented with 10 mM $MgSO_4$. The infected culture was incubated at 37° C on a shaker until cell lysis occurred (approximately 6-8 h). The supernatant fluids with phage particles were collected by centrifugation and phage DNA was purified as described previously.[15]

Transformation and plasmid DNA isolation. Purified pUC19holys plasmid DNA (10 ng) (kindly supplied by B. Henrich of Universitat Kaiserslautern, Germany)[13] was used to transform, by electroporation, *E. coli* strain DH5α. Purified DNA was mixed on ice with electroporation competent cells and transformation was performed in a Bio-Rad Gene Pulser (Bio-Rad Laboratories, Hercules, CA) using a 0.2 cm electroporation cuvette, as described previously.[5] The parameters of the Gene Pulser were as follows: 2.5 kV, 25µF capacitance, and resistance at 200 Ω in parallel with the sample; one electric pulse was applied to the sample. The transformed cells were diluted with 1 ml of SOC (LB plus 10 mM $MgSO_4$ and 0.2% glucose), and incubated on a shaker at 37°C for 1 h. Aliquots of the transformation mixture were spread on LB agar supplemented with ampicillin (100 µg/ml).

Individual colonies were inoculated into LB containing ampicillin at 100 µg/ml and incubated at 37°C in a shaker for 12-14 h. Plasmid DNA was isolated from 1 ml of bacterial culture as described previously.[15] To obtain a large amount of plasmid DNA, a 200-ml culture was prepared. Plasmid DNA

was isolated[15] and further purified by cesium chloride-ethidium bromide density gradient centrifugation at 65K rpm for 5 h in a Beckman vTi80 rotor (Beckman Instruments Inc., Fullerton, CA).

Southern blot analysis. Purified bacteriophage genomic DNA was digested with various restriction endonculeases and separated by agarose gel electrophoresis. DNA fragments were transferred to Genescreen (New England Nuclear Research Products, Boston, MA) in 0.4 M NaOH. The membrane was prehybridized at 42°C for 2 h in a solution containing 5X SSC (1X SSC is 0.15 M NaCl and 0.015 M sodium citrate), 50% formamide, 10% dextran sulfate, 1X Denhardt's solution, 1% SDS, 0.5% sodium pyrophosphate, and denatured herring sperm DNA (200μg/ml) (Boehringer Mannheim Biochemicals, Indianapolis, IN). DNA labeled with ^{32}P-dCTP (New England Nuclear Research Products) were denatured at 100°C for 10 min and added to the membrane. Routinely, hybridization was at 42°C overnight and post hybridization washes under conditions of high (65°C in 2xSSC and 0.1% SDS) or low (40°C in 5xSSC and 0.1% SDS) stringency were as described previously. [7, 15] Membranes were allowed to air dry and exposed to Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY).

RESULTS

Optimal conditions of phage lysate preparation. A confluent lawn of lysed bacterial cells was observed on an agar plate consisting of bacteria infected previously with 10^2 phage particles. Approximately 1.9×10^9 phage forming units per mililiter (pfu/ml) was obtained in the phage lysate eluted from the agar surface. At least 3 mililiters of phage lysate (10^9 pfu/ml) were obtained from one agar plate with confluent cell lysis. Thus, this plate amplification procedure was adopted as a routine method to generate preparative amounts of phage lysate stocks. To determine the optimal conditions for infecting bacteria in a broth culture, *A. viscosus* MG-1 (2×10^8 cells/ml) was infected with various amounts of ϕ63 phage particles. As shown in Table 2, the most efficient infection ratio of phage particles to bacterial cells was 10:1, respectively. Under the indicated infection condition, a clear lysate was obtained after overnight incubation. Approximately 45 μg of ϕ63 DNA was isolated from the supernatant fluid of a 100 ml infected culture.

Restriction endonuclease analysis of phage 63. No recognition sequences for the restriction endonucleases *Bam*HI, *Hind*III or *Dra*I, were detected in the genome of ϕ63. However, unique DNA fragments were obtained by digestion with *Eco*RI, *Kpn*I, *Sst*I, *Sma*I, *Xho*I, *Bgl*II, *Bst*XI, *Bcl*I, *Sph*I and *Sal*I (Table 3, Figure 2). Results from these studies indicated that phage ϕ63 had a genomic size of 18±2 kb, which was consistent with results from the previous study.[7] To assist in determining a physical map of the genome of ϕ63, purified phage DNA was digested with *Eco*RI to completion and the 10-kb *Eco*RI DNA fragment was analyzed further by digestion with various restriction endonucleases (Table 4, Figure 2). A partial physical map of phage ϕ63 genome (Figure 3) was established based on restriction analyses of the entire genomic DNA and the 10-kb *Eco*RI DNA fragment (Tables 3 and 4).

Molecular cloning of ϕ63 DNA. The three (10-, 2.0 and 1.8 kb) *Eco*RI DNA fragments derived from ϕ63 were used in a pilot study for subcloning into the expression vector, λgt11. Plaques were observed when *E. coli* Y1088 was used as the recipient host strain. Only a few recombinant clones were obtained that consisted of the 10-kb *Eco*RI DNA fragment in λgt11 (Table 5). In contrast, 35% and > 80% were recombinant clones in the libraries consisting of the 1.8-kb or 2.0 kb *Eco*RI DNA fragments, respectively (Table 5).

Several recombinant plaques from each library were selected at random and DNA isolated from these clones was digested with *Eco*RI. The digested DNA fragments were examined by agarose gel electrophoresis. Results of these studies indicated the presence of the expected inserted DNA fragment in the respective recombinant clones.

Identification of a putative ϕ63 lysin gene. To examine whether genes that encoded cell lysis activity might be present on the *Eco*RI DNA fragments, aliquots of the in-vitro packaged recombinant phages were used to transfect *E. coli* MC4100. Since this host strain had a defective cell lysis gene, plaque formation would be restored, by complementation, by recombinant packaged lambda phage carrying the lysis genes. However, no plaques were observed.

Since phage lysin genes are highly conserved,[17, 18] a pilot study also was initiated to examine the presence of DNA sequences in ϕ63 that might be homologous to the *Lactobacillus gasseri* ϕadh lysin gene. The plasmid pUC19*holys*, carrying the 342 basepair (bp) *hol* gene and the 951 bp *lys* gene for the *Lactobacillus gasseri* bacteriophage ϕadh was obtained from B. Heinrich.[13] A 1.4 kb *Eco*RI DNA fragment, containing the hol and lys genes was isolated, labeled with ^{32}P-dCTP, and used in Southern blot hybridization to ϕ63 genomic DNA digested with various restriction endonucleases. Although hybridization signals were detected under conditions of moderate stringency (Figure 4), the DNA probe appeared to hybridize to the entire genome of ϕ63, implying these signals were non-specific. These data

indicated insignificant sequence homology between the putative lysin gene of phage ϕ63 and the lysin genes from *Lactobacillus* ϕadh at the nucleotide level.

DISCUSSION

Data from a previous study[7] show that both lytic and temperate bacteriophages are present in human dental plaque. Preliminary analyses indicate that some of these phages have broad host range characteristics. A partial physical map of the lytic phage ϕ63 has been established based on data from restriction endonuclease analyses of the genomic DNA and selected DNA fragments from this phage. Moreover, results of molecular cloning of some of the ϕ63 DNA fragments onto an *E. coli* expression vector indicated that they were stably maintained in the cloning host strain *E. coli* Y1088. The availability of the resultant recombinant clones from this study should greatly facilitate further analysis of the ϕ63 genomic DNA.

Of the three *Eco*RI DNA fragments derived from ϕ63 used in the cloning experiment, a significantly higher percentage of recombinant clones was obtained that contained the 1.8- or 2.0-kb DNA insert (Table 5). In contrast, only a few recombinants were obtained containing the 10-kb insert. These results were consistent with the expected limitation (not to exceed DNA fragments > 7kb) of the cloning vector, λgt11. A different expression vector accommodating larger inserted DNA fragments may be needed for future molecular cloning of the 10-kb *Eco*RI DNA fragment.

A review of the literature indicates that most bacteriophages of both gram-positive and gram-negative bacteria express at least two phage-encoded genes that exhibit call lysis activity.[17] Since the lysins are small membrane proteins, a preliminary attempt was initiated in this study to examine whether the 2.0- or 1.8-kb *Eco*RI DNA fragment might contain the putative lysin genes for ϕ63. However, no positive complementation as indicated by the presence of clear plaques was observed in *E. coli* MC4100 following transfection with recombinant phages containing these DNA fragments. It is possible that the complete lysin gene(s) of ϕ63 is not present on these DNA fragments. Alternatively, the lysin gene(s) may contain internal *Eco*RI recognition sites. Consequently, no functional lysis activities will be

expected from recombinant clones containing any inserted *Eco*RI DNA fragments. Further studies will be needed to prepare a genomic library of ϕ63 digested with a different restriction endonuclease. Alternatively, partial digestion of ϕ63 DNA with *Sau*3AI that result in the generation of DNA fragments of various sizes at random, would be a suitable strategy.

Whereas sequence homology is observed among lysin genes of several bacteriophages from many bacterial species,[13, 17, 18] no significant hybridization signals were detected in the genome of ϕ63 that were homologous to the *Lactobacillus gasseri* ϕadh lysin gene. The experimental conditions employed in this pilot study may not have provided the optimal condition for hybridization duplex formation. In this regard, a reduction in the stringency of hybridization condition may be worth pursuing. On the other hand, as Heinrich noted in his study of the *L. gasseri* ϕadh lysin gene, similarity in gene function may be correlated with homology in primary nucleotide sequence.[13] The lack of detectable sequence homology would be consistent with notion that the lysin genes of *actinomyces* phages and those from *lactobacillus* phages were evolved from different ancestors. Indeed, results of studies of *Actinomyces* fimbriae have indicated that the fimbrial genes of these bacteria are derived from an ancestor distinct from those of other prokaryotic proteins.[19] Clearly, further studies of this and other *Actinomyces* phage are needed and the results should advance our knowledge concerning various genes/gene functions of bacteriophages from these gram-positive bacteria.

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Table 1. Bacterial strains, plasmids and bacteriophages

Bacteria/plasmids/phages	Genotype/remarks[a]	References/Sources
Actinomyces viscosus		
MG-1	Isolated from human dental plaque, host strain of bacteriophage ϕ63	Yeung and Kozelsky, 1997
E. coli		
MC 4100	Sm^r	American Tissue Culture Collection
Y1088	Ap^r	Stratagene
DH5α	Ap^s	Gibco-BRL
Plasmid		
pUC19holys	Contains the *Lactobacillus gasseri* bacteriophage ϕadh *hol* and *lys* genes	Henrich *et al.*, 1995
Bacteriophages		
λgt11	λ*lac5* Δ*shnd*IIIλ2-3 *sr*Iλ3° cIts857 *sr*Iλ4° *nin5 sr*Iλ5°Sam 100	Huynh *et al.*, 1984
ϕ63	*Actinomyces* specific lytic phage	Yeung and Kozelsky, 1997

[a] Sm, streptomycin; Ap, ampicillin; "r", resistant; "s", susceptible.

Table 2. Total phage DNA yield[a]

Phage particles used for infection (pfu)	Phage DNA (μg)
3.0×10^7	0.150
1.5×10^8	9
3.0×10^8	12
1.8×10^9	30
3.0×10^9	15

[a] Various amounts of ϕ63 phage particles were used to infect 100 ml of early exponential phase cultures of *A. viscosus* MG-1. Phage DNA was isolated from phage particles released to the supernatant fluid of each infected culture.

Table 3. Restriction endonuclease analysis of ϕ63 genome[a]

Restriction Endonuclease	Size of DNA Fragments (kb)							
*Bcl*I	>12	3.0	0.8					
*Bcl*I/*Eco*RI	10	2.0	1.0	0.8	0.4			
*Bgl*II	12	5						
*Bgl*II/*Eco*RI	2.0	1.8	1.0	0.7				
*Bgl*II/*Kpn*I	6.5	4.8	4.8					
*Bgl*II/*Xho*I	5.0	4.5	3.5	2.8				
*Bst*XI	11	3.8	0.8	0.5				
*Bst*XI/*Eco*RI	10	2.0	1.8	1.0	0.7	0.4		
*Eco*RI/*Sst*I	10	1.4	1.3	1.2	0.7	0.6	0.5	0.3
*Eco*RI	10	2.0	1.6	1.2	0.7	0.6	0.3	
*EcoR*I/*Kpn*I	4.6	4.6	2.0	1.6	1.2 0.7	0.7	0.6	0.3
*Kpn*I	9	7.0						
*Kpn*I/*Sal*I	9	6.5	2.8					
*Sal*I	12	4.2						
*Sma*I/*Eco*RI	10	2.0	1.6	1.2	0.7	0.6		
*Sma*I	11	5						
*Sph*I	11	4.4	0.7					
*Sst*I	12	2.3	1.4					
*Xho*I	9	6	3.0					
*Xho*I/*Kpn*I	8.3	5.2	1.6	1.3				
*Xho*I/*Sal*I	7.5	3.5	2.8	1.3				
*Xho*I/*Eco*RI	5.5	2.8	2.8	2.0	1.6	0.9		

[a] Purified genomic DNA (500 ng) was digested with various restriction endonucleases. The digested DNA fragments were separated by agarose gel electrophoresis, stained with EtBr and their sizes determined using DNA markers analyzed on the same gel.

Table 4. Restriction endonuclease analysis of the 10-kb *Eco*RI DNA fragment derived from ϕ63[a]

Restriction Endonuclease	Size of DNA Fragments (kb)			
*Bcl*I	10			
*Bgl*II	10			
*Bst*XI	10			
*Kpn*I/*Sal*I	4.0	3.5	3.0	
*Kpn*I/*Sma*I	6	4		
*Kpn*I	6	4		
*Sal*I	7	3.2	0.3	
*Sma*I/*Sal* I	6.5	3.2	1.2	
*Xho*I	5.0	3.0	1.3	
*Xho*I/*Kpn*I	4.5	1.6	1.3	
*Xho*I/*Sma*I	4.5	2.8	1.2	
*Xho*I/*Sal*I	3.5	3.0	2.1	1.3

[a] Purified genomic ϕ63 DNA (10 μg) was digested with *Eco*RI and the 10-kb DNA fragment was eluted from agarose. The isolated DNA fragment was digested with various restriction endonucleases and analyzed by agarose gel electrophoresis.

Table 5. Molecular cloning of ϕ63 DNA fragments

Recombinant library consisting of λgt11 plus ϕ63 *Eco*RI DNA fragment	Transfection Efficiency[a] (pfu/ml)	Percent[b] recombinant
10 kb	2.6×10^4	8
2.0 kb	2.5×10^6	89
1.8 kb	4.6×10^3	35

[a] Defined as the total number of plaque forming units (pfu)(blue and colorless plaques included) per ml of packaged phages. The recipient host of transfection in these assays was *E. coli* Y1088.

FIGURE LEGENDS

Figure 1. Electron micrograph of an isolated particle of phage ϕ63, negatively stained with 1% phosphotungstic acid. Note that this phage has a polyhedral head and a short tail. Bar = 50 nm.

Figure 2. Restriction endonuclease analysis of purified ϕ63 genomic DNA and the 10-kb *Eco*RI DNA fragment derived from this phage. Approximately 500 ng of DNA were digested with (Lanes 1 through 7 and 9 through 16) *Pst*I, *Bgl*II, *Bst*XI, *Bcl*I, *Xho*I, *Sph*I, *Sal*I, *Sma*I/*Sst*I, *Pst*I, *Bgl*II, *Bst*XI, *Bcl*I, *Xho*I, *Sph*I, *Sal*I, respectively. The digested fragments were analyzed on a 0.7% agarose gel and stained with ethidium bromide. Lane 8 contains the 1 kb DNA molecular marker.

Figure 3. Partial physical map of the *Actinomyces* bacteriophage ϕ63. The estimated genomic size of the circular double-stranded DNA molecule is 18±2 kb. The relative locations of the three *Eco*RI DNA fragments (▨) on ϕ63 DNA (═) that were subcloned onto λgt11, and selected restriction endonuclease recognition sites are indicated.

Figure 4. Detection of a putative lysin gene homologue. Panel A. Purified ϕ63 genomic DNA was digested with (lanes 2 through 11) *Sst*I, *Bst*XI, *Xho*I, *Bcl*I, *Sal*I, *Eco*RI, *Bgl*II, *Kpn*I, *Sma*I and *Sph*I, respectively. Lanes 1 and 12 contain molecular markers. The digested DNA fragments were separated by agarose gel electrophoresis, stained with ethidium bromide and then transferred to GeneScreen. Panel B. The membrane was hybridized to the *Lactobacillus gasseri* ϕadh lysin gene under hybridization conditions of moderate stringency and then exposed to x-ray film.

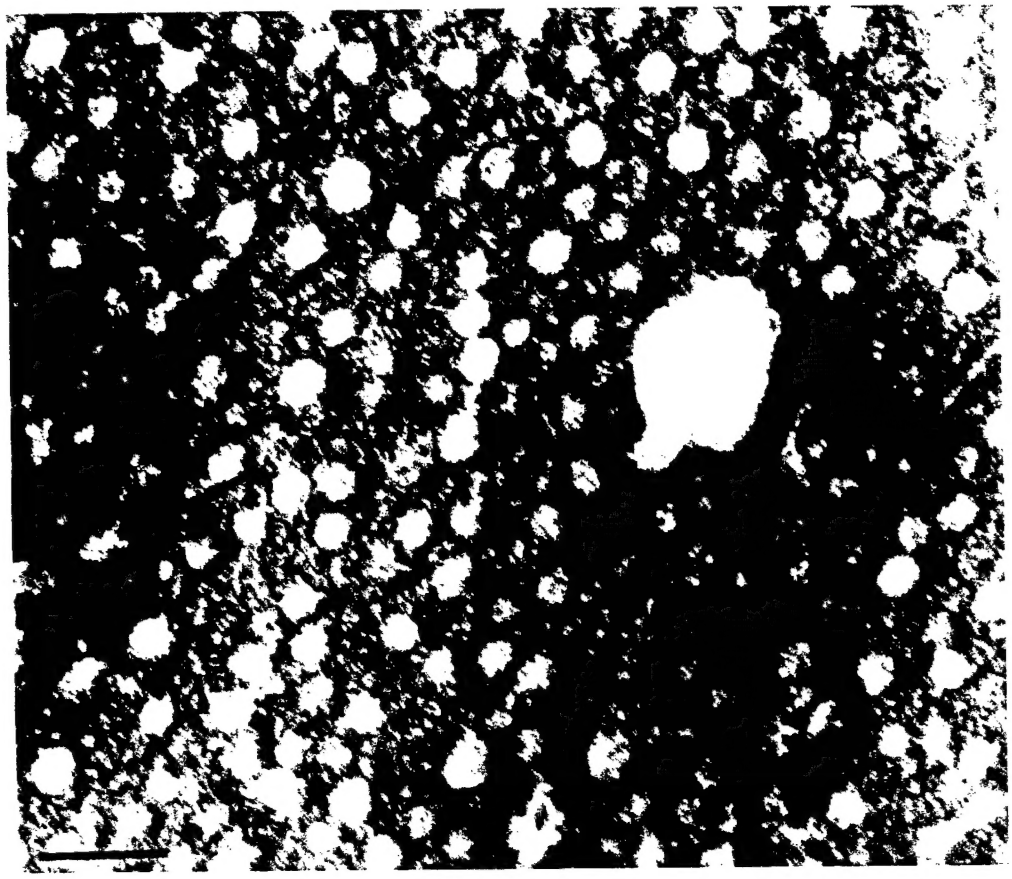


Figure 1

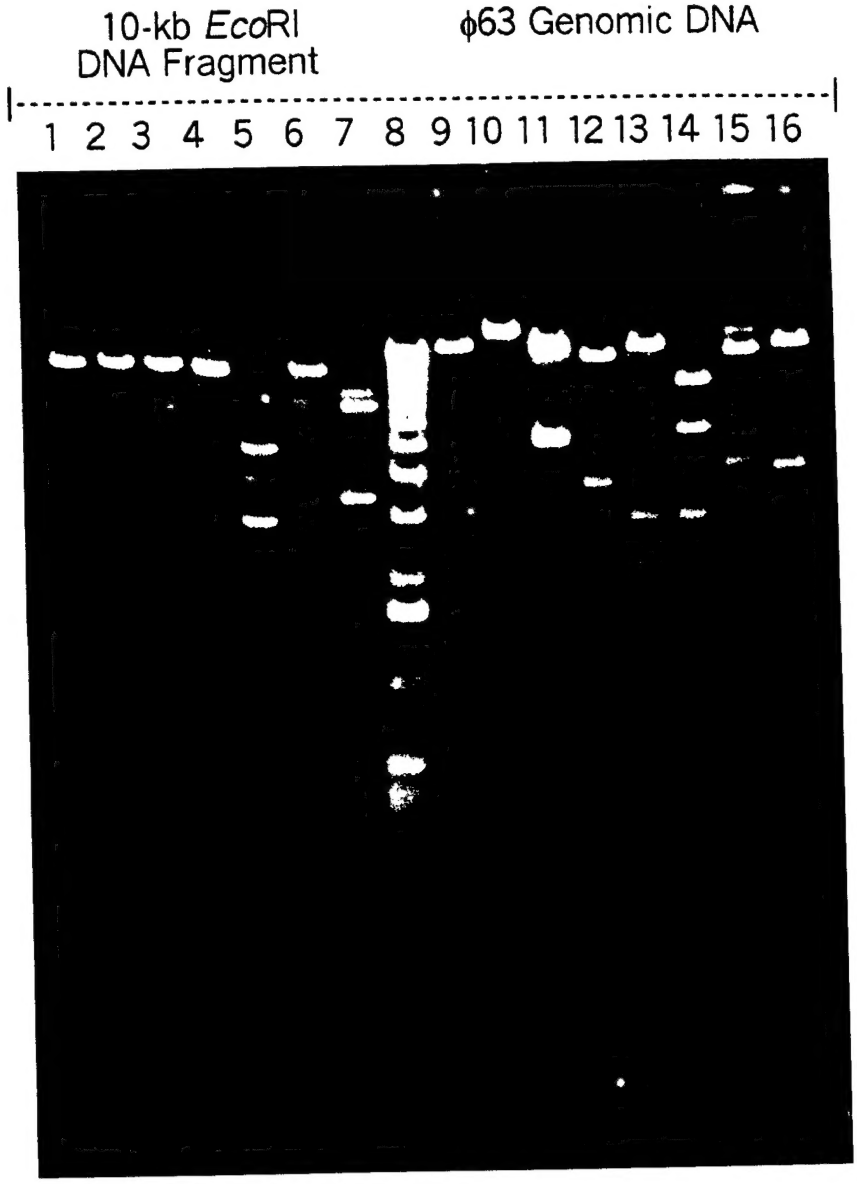

10-kb EcoRI
DNA Fragment
ϕ63 Genomic DNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure 2

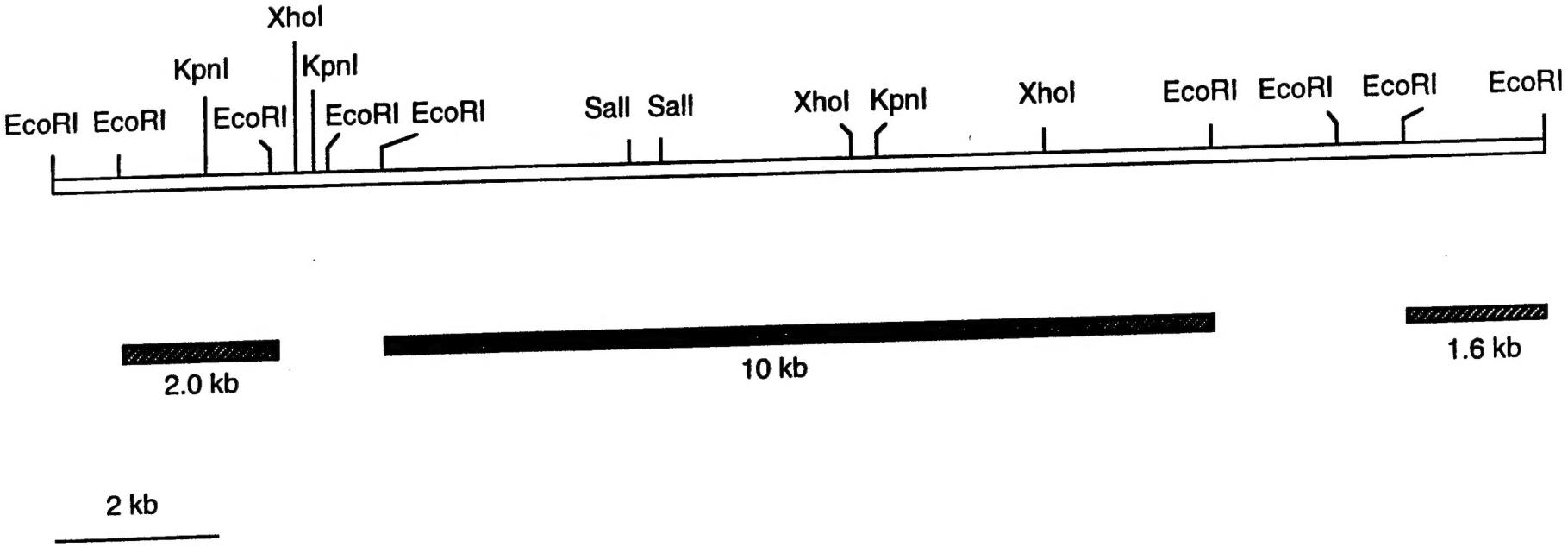
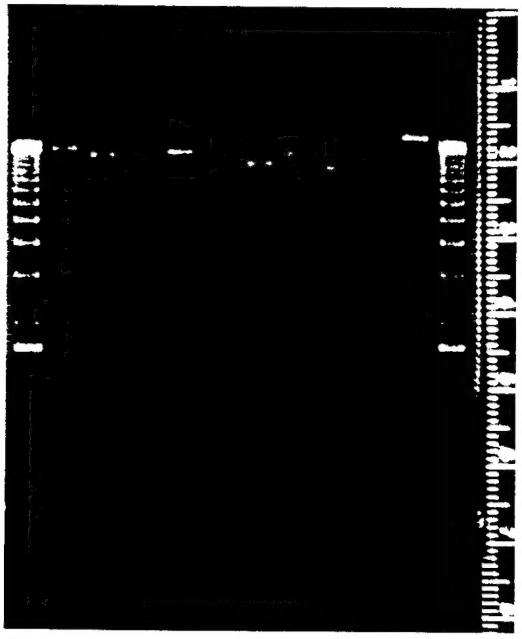

0
XhoI
KpnI
KpnI
EcoRI EcoRI
EcoRI
EcoRI EcoRI
SalI SalI
XhoI KpnI
XhoI
EcoRI EcoRI EcoRI
EcoRI
2.0 kb
10 kb
1.6 kb
2 kb

Figure 3

A

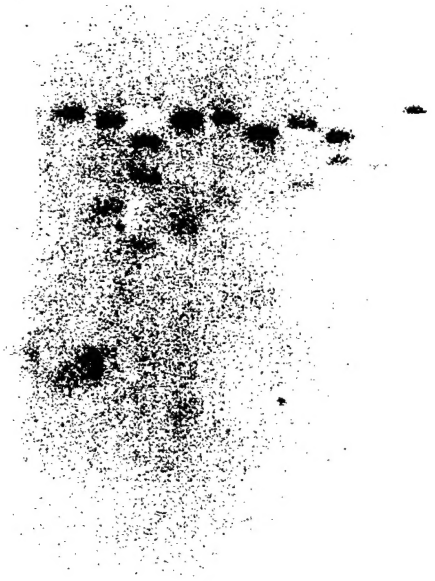
1 2 3 4 5 6 7 8 9 10 11 12



EtBr Stained
DNA fragments

B

1 2 3 4 5 6 7 8 9 10



Southern Blot
Hybridization Profile

Figure 4